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MOTILE CHARACTERISTICS OF 9+2 AND 9+1 FLAGELLAR AXONEMES OF *CRITHIDIA ONCOPELTI*

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Summary

Crithidia oncopelti flagella were progressively solubilized by exposure to solutions containing the detergent Nonidet P42. During the course of the exposure, demembranated flagella were obtained that first had 9+2 axonemes, then 9+1, followed by 9+0. Finally, cylinders of A microtubules were obtained. The 9+2 and 9+1 flagella could be reactivated, indicating that the central complex does not have to be intact to allow wave propagation in this preparation. 9+0 axonemes could not be reactivated. Fourier analysis showed that the waveforms of the 9+2 and 9+1 axonemes were essentially the same, conforming to a meander shape. This shape is significantly different from the arc-line waves seen on *in vivo* flagella and indicates that (a) the membrane plays a significant role in the regulation of this parameter and (b) the passive elastic resistance of the microtubules in demembranated flagella has an important influence on bend patterns. The responses of 9+2 and 9+1 demembranated flagella to ATP were essentially the same, demonstrating that the extraction process had not affected the dynein–tubulin interaction. The central complex in *Crithidia* may be responsible for controlling the direction of wave propagation.

Introduction

The demembranated flagellum of *Crithidia oncopelti* can be reactivated after removal of one of the central microtubules, together with its associated central structures and radial spokes (Marchese-Ragona & Holwill, 1980; Marchese-Ragona *et al.* 1983). When both central microtubules have been removed from this flagellum, reactivation has not proved possible. In two cell types, *Anguilla* sperm (Baccetti *et al.* 1979; Gibbons *et al.* 1985) and *Chlamydomonas* mutants (Brokaw & Luck, 1985), intact flagella with nine peripheral doublets but containing no

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central microtubules are known to be motile. Other motile flagella lacking the central pair, but with different peripheral microtubular arrangements (3+0, Prensier *et al.* 1980, and 6+0, Schrével & Besse, 1975) have also been observed. The flagellum of *C. oncopelti* is the only flagellum from which central structures can be removed chemically without destroying the capacity for movement. The preparation thus has great potential for the elucidation of the role in motility of the central apparatus of flagella.

Although the central complex does not form an integral part of the mechanism required for axonemal motility, observations on *Chlamydomonas* flagella and *Anguilla* sperm suggest that this structure determines the symmetry of bending, being associated with the sensitivity of the organelles to calcium ions (Brokaw *et al.* 1982; Gibbons *et al.* 1985). The motile response of an axoneme to the removal of a central microtubule and radial spokes is therefore of importance, since it may provide more information about the function of these structures in the intact axoneme. If the active binding of radial spokes to the central complex is a source of resistance leading to the conversion of sliding into bending in the axoneme (Warner & Satir, 1974), a change in wavelength, amplitude or frequency might be expected to accompany the degradation. Mechanical considerations alone suggest that the removal of some of the axonemal structure would lead to an observable modification of the flagellar beat parameters, if only because the gross elastic properties of the system would be altered. Since flagellar bending involves the conversion of chemical energy into mechanical energy, a study of the chemical kinetics of the system might be expected to reveal functional changes in the dynein–tubulin interaction caused by degradation of the active regions of either, or both, of these components.

To assess the role of the central complex in motility, we report here the results of a series of experiments in which the wave parameters of axonemes with intact central complexes are compared with those of axonemes where one of the central microtubules is degraded. Our results support earlier conclusions (Marchese-Ragona & Holwill, 1980; Marchese-Ragona *et al.* 1983) that the central complex is not necessary for motility and has no significant effect on wave shape.

Materials and methods

Preparation of 9+1 axonemes

Cells from a 5- to 6-day-old culture of *Crithidia oncopelti* were harvested by gentle centrifugation and rinsed three times in a wash solution containing $100\,\text{mol}\,\text{m}^{-3}$ ($=1\,\text{mmol}\,\text{l}^{-1}$) $MgCl_2$ adjusted to pH 7·0 with mercaptoacetic acid (thioglycolic acid). Cells were demembranated using 0·1 % (w/v) of the non-ionic detergent Nonidet P42 in the wash solution. The demembranation was performed with $1{\cdot}0\,\text{cm}^3$ of the Nonidet solution for every 1·0 mg of cell protein (measured by the method of Lowry *et al.* 1951), at a temperature of 18°C for 25 s. The preparation was then cooled rapidly to 0°C and stored at this temperature for various intervals (see Results). To determine the effects of divalent-cation

chelating agents on motility, EGTA or EDTA at a concentration of 5 mol m^{-3} was added to the wash, extraction and reactivation solutions.

Reactivation of axonemes

Axonemes were reactivated at different ATP concentrations, with the magnesium concentration maintained at 4·5 mol m^{-3}. At the ATP concentrations used (within the range 10^{-3} to 10^{-1} mol m^{-3}) an almost constant proportion (approx. 80 %) of the ATP existed as $MgATP^{2-}$ while the magnesium ion concentration remained essentially constant (Storer & Cornish-Bowden, 1976).

The reactivated samples of axonemes were observed using dark-field optics on a Zeiss WL research microscope, and flagellar images were recorded using a Jackson high-speed (100 frames s^{-1}) video system (Jackson Electronics, Newark, Nottinghamshire, UK). Beat frequencies above 8 Hz were measured using a stroboscopic technique, whereas those below 8 Hz were determined by counting the number of waves propagated by a flagellum in 10 s. Suitable flagellar images were traced from the video monitor onto transparent plastic, and Cartesian coordinates generated using a bit pad. Samples remained active for a period of about 5 min, in which time 10–20 organisms were examined.

Wave-shape analysis

For wave-shape analysis (described in detail by Johnston *et al.* 1979), raw Cartesian coordinate data were converted into the more convenient $\phi(s)$ format, where ϕ is the angle between an arbitrary reference direction and the tangent to the flagellum at a distance s measured along the flagellum from one extremity. Because of asymmetries in the flagellar waves, the images are divided into quarter-waves using turning-points in the $\phi(s)$ representation, and a Fourier analysis performed on each quarter-wave, assuming it to be part of an infinite wave train. A Fourier series is used to express ϕ in terms of s:

$$\phi(s) = \sum b_{(2n-1)} \sin\left[(2n-1)\frac{\pi s}{2S}\right], \tag{1}$$

where S is the length of the flagellum and π has its usual meaning. The coefficients $b_{(2n-1)}$ are given by the integral:

$$b_{(2n-1)} = \frac{2}{S}\int_0^S \phi(s)\sin\left[(2n-1)\frac{\pi s}{2S}\right] ds, \tag{2}$$

and for a particular wave shape the set of coefficients is unique. Analytical waves of relevance to the present discussion are the arc-line (i.e. a wave formed by joining circular arcs by straight lines) and the meander (the curve of minimum energy adopted by an elastic rod when its freely hinged ends are separated by a distance less than its length) since both have been reported for flagella (e.g. Brokaw, 1965; Johnston *et al.* 1979; Hiramoto & Baba, 1978). Coefficients for these waves and a sine wave are shown in Table 1.

Table 1. *Fourier coefficients for quarter-wave sections of some theoretical curves*

	Harmonic						
Wave shape	b_1	b_2	b_3	b_4	b_5	b_6	b_7
Sinusoid	100	13·5	3·9	1·0	0·3	0	0
Arc-line	100	−10·4	3·26	−1·31	0·53	−0·16	−0·04
Meander	100	0·3	0	0	0	0	0

For each wave the ratio λ_s/λ is 1·35, where λ_s and λ are, respectively, the wavelengths measured along the curve and along its axis.

The arc-line curve contained 92 % circular arc.

For a sine-generated curve, all coefficients except b_1 are zero.

Electron microscopy

Samples were prepared for thin-section electron microscopy by fixation in 2·5 % (v/v) glutaraldehyde in 100 mol m^{-3} sodium phosphate buffer at pH 7·0 for 2 min. The cells were then washed three times in phosphate buffer, postfixed with 1·0 % (w/v) OsO_4 in buffer, washed a further three times in buffer solution, and suspended in a fibrin clot (Furtado, 1970). Dehydration was performed in a graded series of ethanol, and was followed by embedding in SPURR resin (EM scope Laboratories Ltd, Kingsworth Industrial Estate, Wotton Road, Ashford, Kent, UK). Silver to gold thin sections were collected on an uncoated 400-mesh copper grid and stained in the dark for 60 min in a 50: 50 ethanol: water mixture containing 1·0 % (w/v) uranyl acetate, followed by Reynolds lead citrate for 3·0 min. Specimens were examined on a transmission electron microscope (Philips 300 or Jeol 100C) operated at 80 kV with a 30 μm objective aperture. All micrographs were taken near optimum defocus.

Results

Structural degradation

Storage of *Crithidia* cells in demembranation solution at 0°C results in progressive degradation of the axoneme (Marchese-Ragona & Holwill, 1980) to an extent which depends on storage time and on concentration of detergent relative to protein mass (Marchese-Ragona *et al.* 1983). At higher concentrations than the optimum (see Materials and methods) axonemes are degraded more rapidly, but the resulting preparation has poor reactivation qualities.

When extraction was performed in the absence of a chelating agent (i.e. the standard demembranation solution), solubilization of the central microtubules proceeded as shown in Table 2 and Fig. 1A,B. The axonemes exhibited four distinct axonemal configurations which could be classified according to the pattern of microtubules remaining. The four categories occurred in a specific temporal sequence starting with the 9+2 configuration of the intact axoneme (Fig. 1A). The sequence of degradation began with the loss of one central microtubule to give a

Table 2. *Microtubule configurations and motility after various storage times*

Storage time (min)	Microtubule configuration								Motile cells (% ± S.D.)
	9+2		9+1		9+0		A		
	%S	%D	%S	%D	%S	%D	%S	%D	
1	84·0	100·0	14·7	100·0	0	–	1·3	0	93·9 ± 1·2
5	78·9	–	16·9	–	2·7	–	1·5	–	–
10	74·0	–	23·3	–	1·35	–	1·35	–	–
60	21·9	90·5	71·7	86·6	5·21	0	0·83	0	83·2 ± 2·3
180	17·1	–	81·5	–	1·32	–	0	–	–
240	4·0	93·3	78·0	79·6	9·0	48·9	9·0	0·8	13·9 ± 2·1

%S is the percentage of a sample having the appropriate structure at the indicated time. Each estimate was obtained from between 75 and 220 flagella images.

%D is the percentage of outer dynein arms present.

Standard errors for the percentage data are about 5 % of the figures quoted.

Motility data were obtained from observations on 300–400 cells at each time indicated.

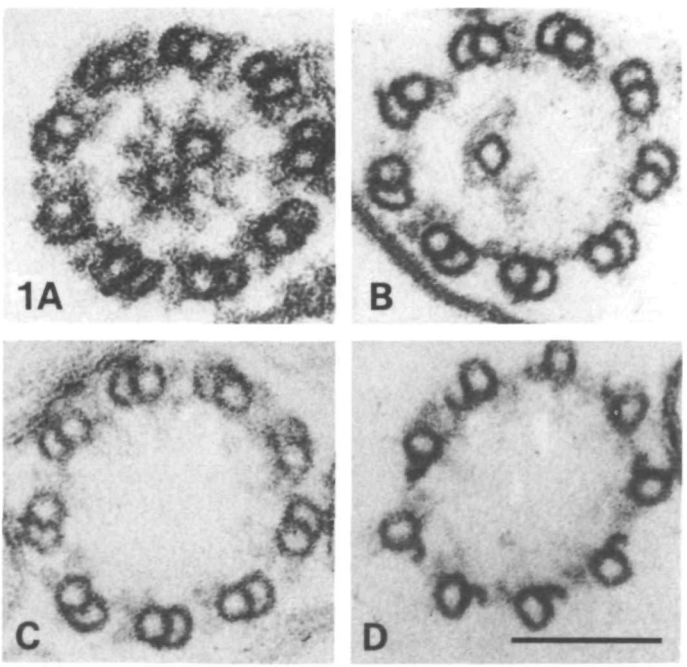

Fig. 1. Electron micrographs of cross-sections of the axoneme of *Crithidia oncopelti* after various storage times in solutions containing no chelating agents. (A) Storage time 1 min: 9+2 structure. (B) Storage time 60 min: 9+1 structure. (C) Storage time 240 min: 9+0 structure. (D) Storage time 2460 min: cylinders of A microtubules. Scale bar, 0·1 μm.

9+1 microtubule configuration (Fig. 1B) followed by the removal of the second central microtubule to leave a 9+0 microtubule configuration (Fig. 1C). Most of the B microtubule of each doublet was then solubilized to produce a cylinder of nine A microtubules each connected to a small portion of the B microtubule; the cylinder was presumably held together by nexin links (Fig. 1D). In some instances, a few A microtubules also showed signs of degradation.

After a 10 min storage period, 74 % of axonemal cross-sections examined by electron microscopy retained the 9+2 microtubular configuration, so samples stored for between 0 and 10 min were used as the control sample of 9+2 axonemes. After 60 min of storage, 72 % of axonemal cross-sections had a 9+1 microtubular configuration, hence samples stored for between 55 and 70 min were used as the 9+1 sample of axonemes. The percentage of cells in a sample which could be reactivated upon the addition of ATP was 90 % after 10 min of storage and 80 % after 60 min of storage. Motility observed at the times specified is thus characteristic of axonemes of the two structural types. After storage times longer than 60 min, the percentage of axonemes with the 9+1 structure increased, but the proportion of motile cells in the sample decreased.

If the wash and extraction were performed in the presence of EGTA or EDTA, the rate of microtubule solubilization was reduced to about one-tenth of its value in the absence of these agents, so that the time required to produce a sample containing predominantly 9+1 axonemes would be in the region of 10 h rather than 1 h. Samples prepared in the presence of EGTA or EDTA also retained the capacity for reactivation for a time which was about 10 times that of samples prepared without the chelating agents. However, the chelating agents induced aggregation of cells to such an extent that it was difficult to observe the behaviour of individual organisms. Samples treated with EGTA exhibited the property of tip-to-base wave propagation, provided they were reactivated with a solution containing the chelating agent within about 15 min of detergent treatment; after this period the direction of wave propagation was from base to tip. A similar effect was seen with EDTA, but the time for which tip-to-base wave propagation was sustained was considerably shorter than for EGTA. Since the time required to degrade one central microtubule was significantly longer than 15 min, no additional information regarding motility could be obtained by adding chelating agents, so all samples were prepared in the absence of EGTA and EDTA, thereby effecting a considerable saving in time.

Between 5 and 10 % of the axonemes had structures which fell between two of the four distinct categories of microtubular configuration. In these cases, axonemes were assigned to the higher level of structural integrity; for example, axonemes with one complete central microtubule and only part of the second microtubule were classified as 9+2 axonemes. Axonemes with only part of one central microtubule were classified as 9+1 axonemes. The proportions of axonemes which fell into these classes after various times are presented in Table 2. This rule of structural assignment, together with the definition that an axoneme would only be recorded as motile if it propagated bends along its entire length,

ensures that, on comparing structure and motility percentages at a given time, the proposition that axonemes lacking a central microtubule are motile can be addressed in the most unfavourable conditions.

The average percentage of axonemes with a 9+1 microtubular configuration after a 1 h treatment period was 71·7 % (maximum, 79·3 %; minimum, 65·7 %) on six separate preparations. In all the preparations incubated for 60 min, 80–90 % of axonemes, as estimated by visual counting, were capable of propagating bends along the total length of the flagellum when reactivated. All transverse sections of 9+1 axonemes examined had lost the central sheath projections and between three and five of the radial spokes that were associated with the solubilized central microtubule. The complement of dynein arms on the peripheral doublets had been reduced by about 10 %.

Comparison of waveforms

In both groups of cells two distinct types of waves were observed. On freely swimming cells the axonemes propagated three-dimensional waves which became two-dimensional when the organelle came in contact with the slide.

Both 9+2 and 9+1 samples showed a variation in waveshapes among cells, but throughout the range of ATP concentrations used all reactivated axonemes conformed roughly to a basic waveform of between one-and-a-half and two wavelengths per flagellum with wave parameters as shown in Table 3. An objective comparison of the two-dimensional waveforms on the two structural types of flagella, at an ATP concentration of 25 mmol m^{-3}, was made using Fourier techniques (see Materials and methods). For the 9+2 sample, 50 flagella centrelines were traced from nine different cells, giving a total of 354 quarter-wave sections whereas, for the 9+1 sample, 28 flagella centrelines were traced from seven cells for a total of 202 quarter-wave sections. From these data the means and standard errors of the first seven coefficients of the best fitting Fourier harmonic series were calculated (Table 4).

Response to ATP

Beat frequencies of reactivated demembranated cells were analysed as mean values over 5-min periods elapsed since demembranation, at the different ATP concentrations (Table 5). Representative data from Table 5 show a slight decrease

Table 3. *Mean bend amplitudes and wavelengths on reactivated axonemes of* Crithidia oncopelti

	Axonemal configuration	
	9+2	9+1
Mean amplitude ± S.D. (μm)	5·2 ± 2·1	4·3 ± 2·0
Mean wavelength ± S.D. (μm)	14·6 ± 2·5	13·1 ± 2·3
Sample size (cells)	50	28

ATP concentration 25 mmol m^{-3}.

Table 4. *Fourier coefficients for quarter-wave sections of reactivated axonemes*

Microtubule configuration	Harmonic						
	b_1	b_2	b_3	b_4	b_5	b_6	b_7
9+2	100	−1·26 ± 1·08	0·58 ± 0·64	−0·33 ± 0·46	0·19 ± 0·34	0·11 ± 0·36	−0·03 ± 0·42
9+1	100	−1·95 ± 1·56	0·78 ± 1·06	−0·41 ± 0·76	0·09 ± 0·42	0·21 ± 0·41	0·13 ± 0·34

Sample size: 9+2, 50 cells, 354 quarter-wave sections; 9+1, 28 cells, 202 quarter-wave sections.
Values ± standard errors.

Table 5. *Beat frequencies of reactivated* Crithidia oncopelti *flagella at various ATP concentrations and storage times*

	Storage time (min)	ATP concentration (mmol m^{-3})*			
		15	20	40	100
T_1	0–5	1·8 ± 0·4	1·4 ± 0·4	3·3 ± 0·4	8·4 ± 1·0
	5–10	1·3 ± 0·1	1·4 ± 0·3	2·5 ± 0·2	10·5 ± 1·0
	20–25	1·4 ± 0·2	1·2 ± 0·2	2·6 ± 0·4	8·4 ± 1·1
	40–45	1·4 ± 0·2	1·4 ± 0·1	–	–
	50–55	1·2 ± 0·3	1·0 ± 0·2	2·5 ± 0·6	8·4 ± 0·9
	55–60	1·1 ± 0·3	1·4 ± 0·2	2·4 ± 0·7	8·9 ± 1·1
T_2	60–65	1·2 ± 0·3	1·1 ± 0·2	2·5 ± 0·3	–
	65–70	0·9 ± 0·2	1·2 ± 0·2	2·7 ± 0·4	9·7 ± 1·2
	70–75	1·2 ± 0·2	1·4 ± 0·4	2·6 ± 0·4	–
	80–85	–	1·3 ± 0·3	2·4 ± 0·4	–

Frequencies are in Hz, and are mean values ± standard deviation.
Each result is derived from about 20 cells.
T_1 and T_2 are, respectively, times when the axonemal structure is predominantly 9+2 and 9+1.
* 1 mmol m^{-3} = 1 μmol l^{-1}.

in beat frequency with time for each ATP concentration (Fig. 2). For the period when axonemal structures were predominantly 9+2 (T_1 in Table 5) or 9+1 (T_2 in Table 5), the reciprocal of beat frequency showed a non-linear relationship with the reciprocal of ATP concentration (Fig. 3), approximating one linear region at concentrations between 5 and 20 mmol m^{-3} and another linear portion between 20 and 250 mmol m^{-3}.

Discussion

Our results indicate that one of the central microtubules of *Crithidia* flagella is chemically more stable than the other; a property found in several organisms (*Chlamydomonas reinhardii*, Jacobs *et al.* 1968; Jacobs & McVittie, 1970; *Tetrahy-*

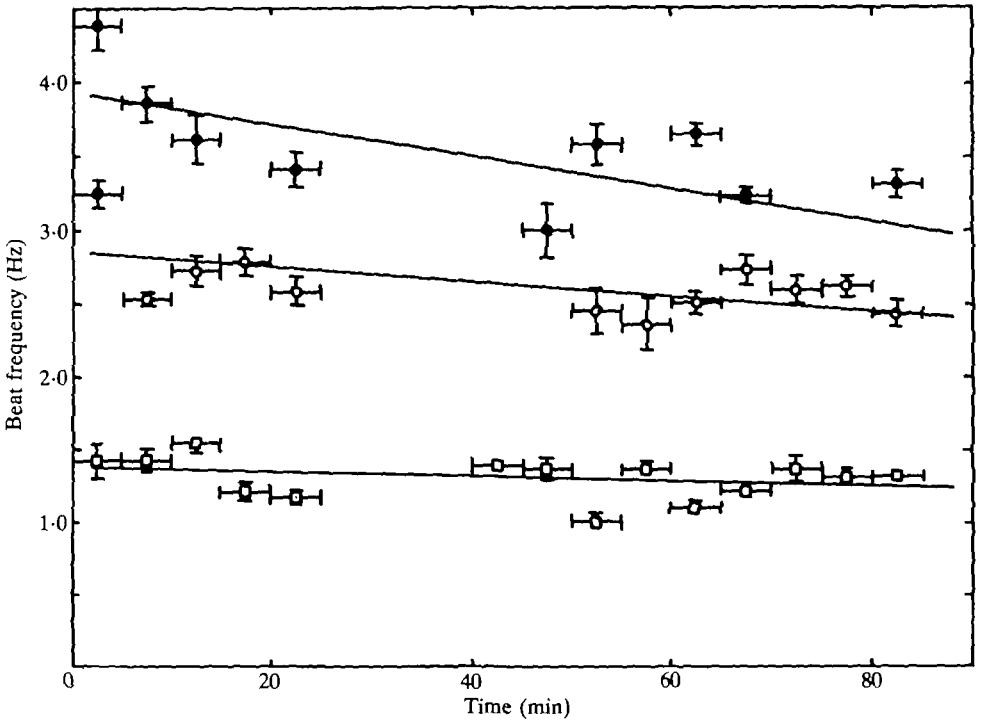

Fig. 2. Variation of beat frequency with time for reactivated *Crithidia* flagella at a number of different ATP concentrations: ●, 60 mmol m^{-3}; ○, 40 mmol m^{-3}; □, 20 mmol m^{-3}.

mena cilia, Chasey, 1969; *Aequipecten irradians* cilia, Linck, 1973*a*,*b*; spermatozoa of the squid *Loligo pealei*, Linck *et al.* 1981). In *Crithidia* we have not been able to identify unequivocally which is the more stable of the two central microtubules, since there is no structural marker to allow the determination of microtubule number; in organisms in which the remaining tubule has been identified, it has proved to be C1 (notation of Linck *et al.* 1981).

The reduced rates of axonemal degradation in the presence of EGTA or EDTA suggest that divalent cations, in particular Ca^{2+}, play an important role in the degradation process. Since calcium ions are known to prevent polymerization of brain tubulin (Scheele & Borisy, 1979) and have been shown here to increase the rate of axonemal degradation, it is possible that the concentration of these ions governs the rate of depolymerization of microtubules of the *Crithidia* axoneme. Microtubules of *Chlamydomonas* are also solubilized by exposure to some detergents (Witman *et al.* 1972), an effect which could possibly be attributed to the presence of calcium ions in the preparation.

The small reduction in frequency that occurs with storage time can be explained in terms of the degradation of dynein arms over this period. Over the initial 1-h period of treatment, there is a reduction of about 10% in the number of dynein

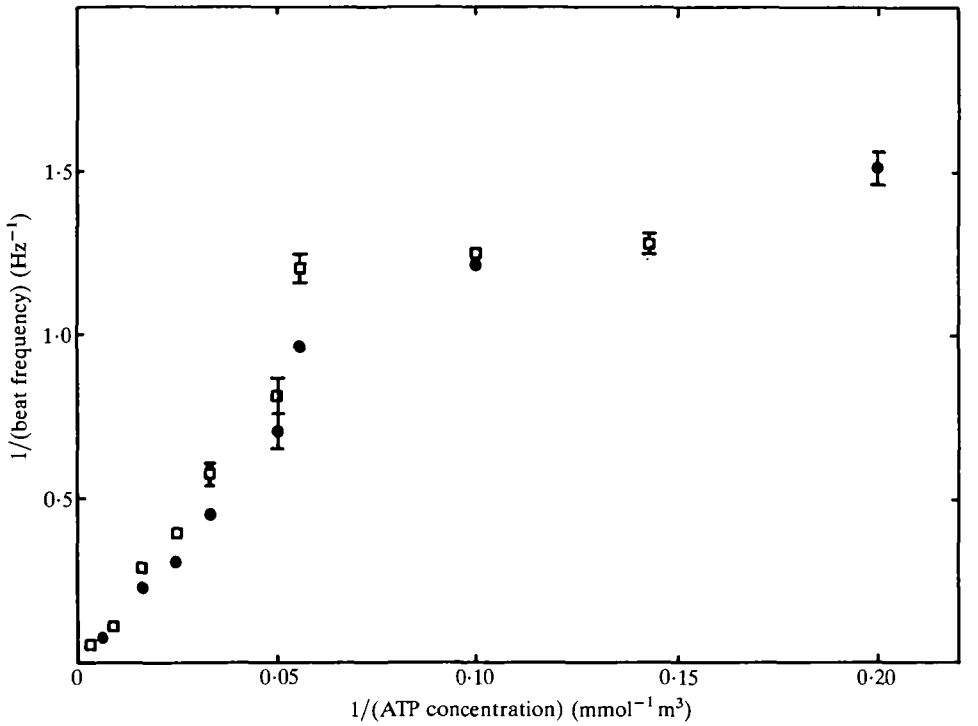

Fig. 3. Double reciprocal plot of beat frequency and ATP concentration for reactivated 9+2 (●) and 9+1 (□) *Crithidia* flagella.

arms (Table 2), and a similar percentage decrease in beat frequency (Fig. 2), suggesting that the beat frequency is related to the number of functional dynein arms in the axoneme. This is consistent with the reduction in frequency which parallels the loss of dynein arms in reactivated sea urchin sperm (Gibbons & Gibbons, 1973). Similar results are reported for mutant *Chlamydomonas* flagella having missing or modified outer arms (Brokaw & Luck, 1985; Kamiya & Okamoto 1985); the beat frequency for these organelles is about 50 % of that of wild-type cells, a reduction corresponding to the percentage loss of dynein arms.

The Fourier coefficients calculated for 9+2 and 9+1 demembranated axonemes (Table 4) represent averaged quarter-waves for the two structural types. Inspection of corresponding coefficients for the two types shows that, within the limits of experimental error, the waveshapes are the same, indicating that the structural degradation had little effect on the mechanism responsible for wave formation and propagation. In particular, this result suggests that the central complex does not make a significant contribution to the passive elastic resistance of the system. This is consistent with recent studies (Holwill, 1989) which indicate that structures other than the microtubules provide a significant resistance to bending.

Although the waveshapes for flagella of the two structural types appear to be identical, comparison of amplitudes and wavelengths (Table 3) suggests that the

more degraded axoneme bears smaller waves. Further experimentation is needed to establish whether the difference is significant.

Apart from b_1, the Fourier coefficients for both the 9+2 and 9+1 axonemes are close to zero, indicating that the flagellar waves are more closely matched by sine-generated waves, or meanders, than by sinusoidal or arc-line waves (compare the coefficients in Tables 1 and 4). The differences between a sine-generated curve and the meander are slight, and would not be detected using the present technique. The waveshape of reactivated flagella is therefore different from that for *in vivo* flagella on forward-swimming *Crithidia* (Johnston *et al.* 1979). In this latter case the bends were characterized as arc-line, with between 76 % and 92 % circular arc; some minor departures from the ideal arc-line curve occur as a result of the mechanical properties of the system. Preliminary studies suggest that waves propagating from base to tip on intact *Crithidia* flagella also appear to be arc-line in form (M. R. Hirons & M. E. J. Holwill, unpublished). A possible explanation for the differing shapes exhibited by intact and reactivated flagella is that the membrane, which is absent in the reactivated cells, exerts some degree of control over the waveshape. Current theoretical models of bend formation and propagation relying only on the mechanical properties of the flagellum and its environment can generate flagellar beat patterns that are an excellent first-order approximation to those observed experimentally (e.g. Brokaw, 1985; Sugino & Naitoh, 1982, 1983). It may be, however, that subtle modifications to the flagellar waveshape are controlled by the membrane. Although the doublets are linked to the membrane in *Crithidia oncopelti* (Marchese-Ragona, 1981), there is little evidence that the membrane could modify the flagellar wavelengths by direct mechanical means (Sleigh & Silvester, 1983). It is more reasonable to suppose that the waveshape is governed to some extent by an active process under cellular control, with ionic fluxes being mediated by the membrane. For convenience of observation and measurement the waveshapes of reactivated 9+2 and 9+1 axonemes were studied at relatively low ATP concentrations. It is possible that higher ATP concentrations may alter the waveshape, but observations on glycerol-extracted *Crithidia* flagella at high and low levels of ATP indicate that there is little alteration in this parameter (Douglas & Holwill, 1972). The meander shape observed on demembranated axonemes suggests that the passive elastic properties of the organelle, which are provided mainly by the peripheral microtubules, play an important role in determining the bend patterns in this case (e.g. Johnston *et al.* 1979).

The difference in waveshape between *in vivo* and demembranated *Crithidia* flagella is at variance with the finding of Weaver & Hard (1985) that the waveshapes of intact and demembranated newt lung cilia were the same. However, these authors based their conclusions on angular measurements of the various phases of the beat cycle, which may not reveal the subtle changes in waveshape reported here. A study of ciliary bend patterns using the Fourier analysis method would be of interest in this regard.

In *Chlamydomonas* flagella, the radial spoke/central sheath interaction is believed to be involved in the control of wave symmetry (Brokaw *et al.* 1982;

Brokaw & Luck, 1985), since mutants lacking these structures appear incapable of propagating highly asymmetric bends. The central complex may therefore be the site of calcium ion action, since variations in Ca^{2+} levels produce changes in wave symmetry of *Chlamydomonas* flagella. This conclusion is supported by the observation that *Anguilla* sperm, which naturally lack the central complex, also have no calcium sensitivity (Gibbons *et al.* 1985). For *Crithidia*, the wave symmetry does not appear to be regulated by the radial spokes, since it is unchanged when some of the spokes, together with the associated central microtubule, appear to be disrupted. However, the response of *Crithidia* flagella to changes in Ca^{2+} concentration is not associated with wave symmetry, but with the direction of wave propagation (Holwill & McGregor, 1976). *In vivo Crithidia*, and certain *in vitro* preparations of the system, maintain proximally directed waves, but reverse the direction of wave propagation when stimulated appropriately. Whereas living cells are able repeatedly to reverse the propagation direction in response to environmental changes, once a reactivated flagellum begins propagating waves from base to tip this propagation direction is maintained; conditions in which the organelle will revert to tip-to-base beating have not been discovered. It has therefore not been possible to test whether the central complex is involved in controlling wave direction.

The non-linearity of the double-reciprocal plots of frequency and ATP concentration (Fig. 3) indicates that the response of the system does not obey Michaelis–Menten kinetics over the entire range of ATP concentrations used in our studies. However, the curves of Fig. 3 can be matched reasonably by two linear sections, corresponding to concentrations in the ranges 5–20 mmol m^{-3} and 20–250 mmol m^{-3}, showing that Michaelis–Menten type behaviour is followed over these ranges considered separately. For each range K_m values are the same for the 9+2 and 9+1 samples, within the limits of experimental error, suggesting that the ATPase kinetics are the same in the two systems. We conclude that the removal of one central microtubule does not alter the enzyme kinetics of the ATP–dynein interaction (which we assume to be reflected by the beat frequency) over the range of ATP concentrations considered.

Other work on *Crithidia oncopelti* (Douglas & Holwill, 1972; McGregor, 1976) and a variety of spermatozoa from marine organisms (e.g. Gibbons & Gibbons, 1972; Brokaw, 1975; Gibbons *et al.* 1985) has shown that at ATP concentrations above about 30 mmol m^{-3} the variation of flagellar beat frequency with ATP concentration (more strictly with MgATP concentration) follows Michaelis–Menten kinetics with K_m values in the range 0·1–0·6 mol m^{-3} (Table 6). Precise agreement between the K_m values for different systems is not expected, since the nature of the enzyme and its chemical environment will vary from one organism to another. Departures from Michaelis–Menten behaviour at ATP concentrations lower than 30 mmol m^{-3} have been noted by Gibbons & Gibbons (1972), Brokaw (1975) and McGregor (1976), but it is not clear whether their results could be matched by two linear regions as discussed above. Reactivated cilia from the newt lung do show a biphasic behaviour similar to that reported for *Crithidia*, and the

Table 6. K_m *values obtained from the behaviour of reactivated flagella*

Organism		[ATP] (mmol m^{-3})*	K_m (mol m^{-3})*	Reference
Crithidia oncopelti	9+2	5–20	0·006 ± 0·004	This study
	9+1	5–20	0·009 ± 0·003	This study
	9+2	20–250	0·14 ± 0·05	This study
	9+1	20–250	0·14 ± 0·1	This study
	9+2	4–100	0·08 ± 0·05	Douglas & Holwill (1972)
	9+2	10–100	0·1 ± 0·03	McGregor (1976)
	9+2	10^2–10^3	0·3 ± 0·1	McGregor (1976)
	9+2	10^3–10^4	0·6 ± 0·2	McGregor (1976)
Sea urchin spermatozoa				
Colobocentrotus atratus		30–100	0·21 ± 0·01	Gibbons & Gibbons (1972)
Lytechinus pictus		150–2000	0·2	Brokaw (1975)
Oseydicebtritys deoressas†		20	0·001	Yano & Miki-Noumura (1980)
Anguilla spermatozoa		0–10^3	0·2	Gibbons *et al.* (1985)
Chlamydomonas				
wild type		0–10^3	0·10 ± 0·02	Kamiya & Okamoto (1985)
wild type†		0–10^3	0·158 ± 0·036	Okagaki & Kamiya (1986)
mutant (no outer arms)		0–10^3	0·060 ± 0·012	Kamiya & Okamoto (1985)
mutant (no outer arms)†		0–10^3	0·064 ± 0·018	Okagaki & Kamiya (1986)
Newt lung cilia		125–500	0·14	Weaver & Hard (1985)
Newt lung cilia		833–2500	0·47	Weaver & Hard (1985)

* 1 mmol m^{-3} = 1 μmol l^{-1}.
† K_m determined from microtubule sliding rate.
In all other cases K_m was determined from beat frequency measurements.

K_m values are also similar (Table 6), but the junction of the two linear regions occurs at about 500 mmol m^{-3}. The present study did not extend to this concentration range, but earlier work (McGregor, 1976) indicates that a third linear region exists for *Crithidia* at higher ATP levels (Table 6).

K_m values derived from measurements of the sliding velocities of microtubules as a function of ATP concentration have been obtained for sea-urchin sperm (Yano & Miki-Noumura, 1980) and *Chlamydomonas* (Okagaki & Kamiya, 1986), and are similar to the values obtained from beat frequency measurements (Table 6). K_m values for microtubule sliding velocities in a bending axoneme correspond closely to those for beat frequencies (Okuno & Brokaw, 1979). The similarity between the K_m values obtained from the frequency and microtubule sliding experiments indicates that the mechanism which converts sliding into bending does not involve a separate enzymic process from that which induces sliding; if different processes were effective, it would be expected that the K_m values derived from beat frequency measurements and sliding velocity observations would differ. It is, therefore, not unreasonable to suppose that the reactions responsible for the production of sliding may also be involved in its

resistance; that is, while some dynein arms are inducing active sliding others are responsible for restricting the sliding and thereby causing bending to occur.

Despite extensive tests to detect a significant difference between the motile characteristics of 9+1 and 9+2 axonemes, the results presented here indicate that they are essentially identical in terms of wave characteristics and enzymic activity. This supports the conclusion reached on the basis of studies on flagellar mutants and deviants that neither the central complex nor the radial spokes is essential for the conversion of sliding into bending, or for wave propagation. The possibility that the central complex is concerned with controlling the direction of wave propagation requires further study, but is a reasonable suggestion in view of the Ca^{2+} sensitivity noted earlier. On the basis of the motile response of *Crithidia* to Ca^{2+}, Holwill & McGregor (1976) note the need for a site of calcium sequestration within the organelle, and the central complex would be an appropriate location. The different waveshapes observed on intact and demembranated flagella suggest that the membrane plays a significant role in determining this parameter.

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